REMARKS

Claims 1-21 and 26-31 are currently pending in the application. Claims 1, 7, 16, and 26 are in independent form.

The priority statement is objected to because it is located below the statement of government support. In response thereto, Applicants have amended the specification to reference priority in the first sentence of the specification. Reconsideration of the objection is respectfully requested.

The drawings are objected to because they include reference characters not mentioned in the description. In response thereto, Applicants have amended the Description of the Drawings section to correctly identify the drawings. The drawings are further objected to because the description of Figure 2 refers to the color red, which cannot be seen in the black and white drawings. In response thereto, Applicants submit herein a color copy of Figure 2 for examination purposes only. Applicants will timely file a petition to accept color drawings and submit three copies of the color figure upon notice of allowance of the claims. Reconsideration of the objections is respectfully requested.

Applicants have noted that Figures 1B-1D are also color drawings, and therefore Applicants have also submitted herein a color copy of Figures 1B-1D for examination purposes only. As with Figure 2 above, Applicants will timely file a petition to accept color drawings and submit three copies of the color figures upon notice of allowance of the claims.

The specification is objected to because it contains an embedded hyperlink and/or other form of browser-executable code. In response thereto, Applicants have

amended the specification to remove the hyperlink. Reconsideration of the objection is respectfully requested.

The specification is objected to because of informalities of misspelling "of" at page 8, line 11. In response thereto, Applicants have amended the specification. Reconsideration of the objection is respectfully requested.

The specification is objected to because of the use of trademarks. In response thereto, Applicants have amended the specification to correct the use of trademarks. Reconsideration of the objection is respectfully requested.

Claims 16 and 17 stand provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-4, 8-9, and 12-13 of copending Application No. 10/593,412. In response thereto, Applicants have amended claim 16 to provide structure of the microarray screen to distinguish over the claims of the copending application to remove any double patenting issues. Reconsideration of the rejection is respectfully requested.

Claims 1-15 and 26-31 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Specifically, the Office Action holds that claim 1 is drawn to a "screen". The device of claim 1 comprises animal cleavage stage embryos and a detecting means for detecting changes in gene expression. The claim does not provide a functional or structural relationship between the embryo and the detecting means. The specification does provide clarity in that the device used in the instant specification is a microarray containing cDNA sequences. The microarray of the instant specification is not brought into physical contact with the embryos. Rather, RNA is isolated from the embryos and subsequently hybridized to

the microarray. The Office Action holds that the metes and bounds of the claimed device are unclear. The same reasoning is applied to claims 7 and 26.

In response thereto, Applicants have amended independent claims 1, 7, 16, and 26 to require that the screen includes detecting means for detecting changes in gene expression, wherein the detecting means includes animal cleavage stage embryo hybridizing means for hybridizing RNA probes generated from chemically treated animal cleavage stage embryos. This amendment provides necessary structure to the screen and provides a relationship between the screen, the detecting means, and the embryos. Specifically, the detecting means include the animal cleavage stage embryo hybridizing means, i.e. the cDNA that corresponds to RNA extracted from the embryos. The specification is fully supported for such a screen, as detailed on pages 11 and 12 as well as the Materials and Methods section of the Examples. Reconsideration of the rejection of claims 1-15 and 26-31 is respectfully requested.

Claims 1-15 and 26-31 stand rejected under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for a microarray device for detecting the effect of a chemical on gene expression, does not reasonably provide enablement for any other device, especially a device containing an animal embryo of any kind. The specification does not enable any person skilled in the art to make and use the invention commensurate in scope with the claims and undue experimentation would be required.

In response thereto, Applicants have amended the independent claims as described above to require that the screen includes detecting means for detecting changes in gene expression, wherein the detecting means includes animal cleavage stage embryo hybridizing means for hybridizing RNA probes generated from

chemically treated animal cleavage stage embryos. Therefore, Applicants have provided the necessary functional and structural relationship between the screen, the detecting means, and the embryos.

The screen of Applicants' invention is not limited to "a microarray device" as the Office Action holds. Rather, the screen "can include any device capable of screening for gene expression in an embryo. An example of such a screen includes, *but is not limited to*, a microarray." Specification, page 8, lines 11-13, emphasis added. It is contemplated that any suitable device that can be used as a screen with the limitations as required by the independent claims would be appropriate. The microarray device detailed by Applicants in the Examples and in the specification is only one embodiment of the screen. One skilled in the art would be able to apply the present invention to any suitable screen.

With respect to the nature of the invention, a component for measuring gene expression has been included in the amended independent claims, i.e. the animal embryo hybridizing means either in cleavage or neurulation stage. The animal embryo hybridizing means allows for cDNA to hybridize with RNA probes, which in turn allows for detection of gene expression levels.

With respect to the breadth of the claims, Applicants have amended the independent claims such that gene expression levels are detected from RNA levels, as hybridization occurs with RNA probes. Therefore, the invention is not directed to detecting protein levels as stated in the Office Action. Further, the screen of the present invention is capable of functioning with <u>any</u> species of organism because of the particular embryo stage used for the hybridization means. It is well known that "embryogenesis initiates upon fertilization of the egg with the first cell division. The early period of embryogenesis *in all animals* is a cleavage stage characterized by

repeated cell divisions without growth resulting in progressively smaller cells in the embryo. Early embryogenesis depends initially on maternally inherited molecules and structures that are gradually replaced by ones synthesized in the embryo." Specification, page 3, lines 5-9, emphasis added. It was an unexpected finding of the present invention "that gene regulation at cleavage stages was extremely sensitive to chemical treatment. Considering that *embryogenesis is highly conserved among animals*, gene regulation studies for animals after chemical treatment of embryos, especially *Xenopus* and mice, at the early embryonic stages have advantages of shorter incubation time after fertilization of the embryos and higher sensitivity." Specification, page 9, lines 16-22, emphasis added. Therefore, it is expected that the screen of the present invention would function with any animal cleavage stage embryo hybridization means.

With respect to the guidance of the specification, the specification teaches how to make and use the device as in the amended independent claims, and as stated above, Applicants have added terms relating the screen to the embryos.

With respect to the predictability and state of the art and amount of experimentation necessary, it is well known in the art how to develop a screen such as a microarray, as detailed on pages 4-6 of the specification. Therefore, from the teachings of Applicants' invention detailing how the particular screen was created, specific guidance would not be required to make the screen using a microarray embodiment. Further, undue experimentation would also not be required to practice the invention. As shown in Appendix I (Table I), Applicants have applied the screen of the present invention to other various chemicals besides the PMA disclosed in the present examples.

Applicants therefore submit that the claims as amended are fully enabled by the specification. Reconsideration of the rejection of claims 1-15 and 26-31 is respectfully requested.

Claims 1-21 and 26-31 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Altmann, et al. Specifically, the Office Action holds that Altmann, et al. teaches *Xenopus laevis* cleavage stage embryos and a microarray comprising cDNA. The cleavage stage embryos and microarray taught by Altmann, et al. would be capable of being used for detecting the effects of chemicals on gene expression, characterize chemicals as toxicants based on the effect of the chemical on gene expression, detecting and measuring the effects of chemicals on gene expression in cleavage stage embryos, and characterizing chemicals as toxicants based on the effect of the chemical on gene expression. Reconsideration of the rejection under 35 U.S.C. § 102(b), as anticipated by Altmann, et al., as applied to the claims is respectfully requested. Anticipation has always been held to require absolute identity in structure between the claimed structure and a structure disclosed in a single reference.

Altmann, et al. is described extensively and distinguished in the specification on pages 3 and 4: "In all animal embyros, one of the first differentiation events is the formation of ectoderm, endoderm and mesoderm cell lineages called the germ layers. Subsequently, gastrulation transforms the spherical blastula embryo into a structure with a hole through the middle that becomes the gut. In *Xenopus*, the germ layers are formed in the blastula, stages 8.5-9, and gastrulation begins in the early gastrula at stage 10. Recently, microarray analysis of gene expression in early *Xenopus laevis* development was reported using microarrays composed of *Xenopus laevis* gastrula cDNAs **(11).** Three investigations were pursued in the study: 1) comparison of maternal versus gastrula transcription, 2) spatially restricted gene

expression in the gastrula embryo and 3) induction of mesoderm germ cells at midgastrula using isolated blastula ectoderm cells treated with the *Xenopus laevis* protein growth factor activin, a known inducer of mesoderm differentiation. Each of these observations provided confirmation of previously known outcomes determined with other molecular technologies. No part of this study involved cleavage stage embryos. The paper concludes with the statement, "based on the success of the prototype arrays, the larger scale arrays should allow the rapid identification of regulated genes under a variety of conditions (page 74)." However, it is important to note that the study was limited to investigating the events of normal embryonic development. Moreover, there is no mention of investigating the impact of chemical treatment on embryogenesis."

While Altmann, et al. refers to "embryogenesis", it does not concern cleavage stage embryos, but rather gastrula stage embryos. The objectives of the Altmann, et al. reference are to discover genes in *Xenopus* expressed at gastrula stage (11) embryos compared to maternal expression. Altmann, et al. compared gene expression from embryos prior to the onset of zygotic transcription at the midblastula transition (stage 6, pre-MBT) to gastrula stage embryos undergoing zygotic transcription (stage 11, post-MBA). Essentially, Altmann, et al. is interested in what genes are new at stage 11, when the embryo has begun to produce its own gene products. The midblastula transition stage 6 embryos were merely used as a control (i.e. gene expression changes were not expected at this stage).

In contradistinction, the presently amended independent claims require animal cleavage stage embryo hybridization means. This stage only contains gene products (mRNA) inherited from the mother. It was unexpected that gene regulation at this stage was extremely sensitive to chemical treatment. Further, at the early embryonic stages, there is an advantage of shorter incubation time after the fertilization of the

embryos and higher sensitivity. Applicants applied chemicals to *Xenopus* embryos at pre-stage 4 (2 hours after fertilization) and harvested at stage 8 (5 hours after fertilization), both pre-stage 4 and stage 8 being in the cleavage stage and before gastrulation, in order to detect changes in gene expression levels. Therefore, since the Altmann, et al. reference does not disclose animal cleavage stage embryo hybridization means as set forth in the presently pending independent claims, the claims are patentable over the Altmann, et al. reference and reconsideration of the rejection is respectfully requested.

Claims 1-21 and 26-31 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Hemmati-Brivanlou, et al. Specifically, the Office Action holds that Hemmati-Brivanlou, et al. teaches microarrays containing *Xenopus laevis* embryonic gene sequences, and cleavage stage *Xenopus laevis* embryos (stage 6). The microarray taught by Hemmati-Brivanlou, et al. would be capable of being used for detecting the effects of chemicals on gene expression, characterize chemicals as toxicants based on the effect of the chemical on gene expression, detecting and measuring the effects of chemicals on gene expression in cleavage stage embryos, and characterizing chemicals as toxicants based on the effect of the chemical on gene expression. Reconsideration of the rejection under 35 U.S.C. § 102(b), as anticipated by Hemmati-Brivanlou, et al., as applied to the claims is respectfully requested. Anticipation has always been held to require absolute identity in structure between the claimed structure and a structure disclosed in a single reference.

Hemmati-Brivanlou, et al., like Altmann, et al. above, is comparing gene expression from "pre-MBT *Xenopus* embryos to post MBT gastrula stage embryos" using microarrays in order to compare maternal mRNA with mRNA that the embryo produces on its own. Paragraph [0118]. The pre-MBT embryos are at stage 6, and the post MBT gastrula stage embryos are at stage 11. Again, Hemmati-Brivanlou, et

al. is concerned about what gene expression has changed *after* gastrulation, not in the cleavage stage, and the same arguments stated above regarding Applicants' invention apply to Hemmati-Brivanlou, et al. as well.

Therefore, since the Hemmati-Brivanlou, et al. reference does not disclose animal cleavage stage embryo hybridization means as set forth in the presently pending independent claims, the claims are patentable over the Hemmati-Brivanlou, et al. reference and reconsideration of the rejection is respectfully requested.

Claims 7-9 and 13-15 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Herwig, et al. Specifically, the Office Action holds that Herwig, et al. teaches a microarray containing zebrafish cDNA clones selected from a representative cDNA library from zebrafish gastrula stage embryos. Reconsideration of the rejection under 35 U.S.C. § 102(b), as anticipated by Herwig, et al., as applied to the claims is respectfully requested. Anticipation has always been held to require absolute identity in structure between the claimed structure and a structure disclosed in a single reference.

Herwig, et al. teaches statistical evaluation of cDNA arrays made from "zebrafish gastrula stage embryos" (page 1). There is no mention of cleavage stage embryos. Again, as stated above, the gastrula stage is much different than the cleavage stage required by the independent claims, and performing a hybridization test at this stage would produce different results than in Applicants' invention. Therefore, since the Herwig, et al. reference does not disclose animal cleavage stage embryo hybridization means as set forth in the presently pending independent claims, the claims are patentable over the Herwig, et al. reference and reconsideration of the rejection is respectfully requested.

The remaining dependent claims not specifically discussed herein are ultimately dependent upon the independent claims. References as applied against these dependent claims do not make up for the deficiencies of those references as discussed above, and the prior art references do not disclose the characterizing features of the independent claims discussed above. Hence, it is respectfully submitted that all of the pending claims are patentable over the prior art.

In view of the present amendment and foregoing remarks, reconsideration of the rejections and advancement of the case to issue are respectfully requested.

The Commissioner is authorized to charge any fee or credit any overpayment in connection with this communication to our Deposit Account No. 11-1449.

Respectfully submitted,

KOHN & ASSOCIATES, PLLC

Kenneth I. Kohn, Reg. No. 30,955
30500 Northwestern Highway
Suite 410
Farmington Hills, Michigan 48334
(248) 539-5050

Dated: May 2, 2007

CERTIFICATE OF MAILING

Express Mail Label No.: EV701295977US
Date of Deposit: May 3, 2007

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" addressed to Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Sheryl M. Fox